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Mouse Embryo Biopsy: Abnormal Development with Trophoblastic Vesicle Formation

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ABSTRACT—Preimplantation mouse embryos at the four- and eight-cell stages were subjected to biopsy and evaluated for abnormal (trophoblastic vesicle) development *in vitro*. A maximum of two blastomeres can be removed from four-cell embryo, whereas four blastomeres can be taken at biopsy from an eight-cell mouse embryo, without significantly increasing trophoblastic vesicle formation. A significant increase in trophoblastic vesicle formation was observed only when the cellular mass per four-or eight-cell embryo was reduced to less than 50%.

INTRODUCTION

Removal of one or more blastomeres from the preimplantation embryo has been proposed for the early diagnosis of genetic disease, detection of chromosomal abnormalities, and the pretransfer detection of transgenic incorporation [1]. The ability to successfully biopsy embryos is dependent upon the cell-stage, number of cells removed and the biopsy technique [1, 2]. Blastomere biopsy has been accomplished at various preimplatation stages of embryonic development from the two-cell to the blastocyst cell [2–6]. Handyside *et al.* [3] have reported the establishment of pregnancy following the uterine transfer of biopsied-sexed human embryos. A barrier to successful utilization of this technique could be abnormal growth patterns. Abnormal growth patterns include trophoblastic vesicles (no inner cell mass, ICM) or multiple blastocoel cavities. While the abnormal growth patterns may be seen in nonmanipulated embryos [7], they are more common in manipulated embryos [5]. Single blastomeres removed from four- or eight-cell mouse embryos uniformly develop into trophoblastic vesicles [8]. It is imperative that the largest possible number of cells be obtained for assay while protecting the developmental integrity of the conception. Whether removal of a single blastomere from a four-cell embryo is detrimental is controversial.

Removal of one blastomere from a four-cell mouse embryo does not significantly reduce *in vitro* or *in vivo* development [2, 9]. However, Krzyminska *et al.* [6] reported that biopsy of four-cell embryos significantly impaired development *in vitro*. Biopsied four-cell embryos often fail to undergo subsequent division or compaction and it has been suggested that the biopsy technique may interfere with development [6]. The dissimilarity between *in vitro* development rates may be attributed to differences in mouse strains, culture media and/or biopsy technique used [2, 10]. Reports on the efficiency of mouse embryo biopsy have suggested that the ideal developmental stage is the eight-cell embryo [2, 6].

The present study was performed to determine the effect of cell-loss (*via* biopsy) and cell-stage on trophoblastic vesicle (no ICM present) formation in the mouse nondifferentiated embryo.

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MATERIALS AND METHODS

Embryos at the one-cell stage were flushed from the excised oviducts of female $B_6D_2F_1/J$ (The Jackson Laboratory, Bar Harbor MA) mice primed with pregnant mare serum gonadotropin (7 IU, Sigma Chemical Co., St. Louis) followed 48 hours later with human chorionic gonadotropin (7 IU, Sigma). Females were bred with fertile $B_6D_2F_1/J$ males immediately after administration of human chorionic gonadotropin. Embryos were collected, manipulated and cultured in Ham's F-10 (Sigma) medium supplemented with 0.3% bovine serum albumin (BSA; Sigma) in an atmosphere of 5% CO_2 in air, 95% relative humidity at 37°C [2].

Mouse embryos were biopsied by the displacement technique at the four- and eight-cell stages as previously described [2]. Briefly, embryos were held in place with a fire polished holding pipette and gentle suction while an opening was made in the zona pellucida with a beveled pipette. The beveled pipette was withdrawn and then reinserted throught a second site, a gentle flow (1–2 psi) of medium injected through the pipette was used to dislodge and displace the blastomeres; subsequently pushing them out of the zona pellucida through the first puncture site. Biopsied embryos were transferred to microdrops (25 μl) of Ham's F-10 medium, under filtered (0.22 μm; Corning, Corning N.Y.) sterilized light mineral oil (Fisher; Fair Lawn, N.J.) in 35 mm tissue culture dishes (Corning). Embryonic development was evaluated after 72 hours in culture. Formation of trophoblastic vesicles, no ICM within a single cellular wall were judged abnormal.

Data were analyzed by the Chi square two-by-two contingency table.

The care and use of the animals were approved by the Animal Care Committee of East Tennessee State University.

RESULTS

A total of 527 nondifferentiated mouse embryos were collected from 30 mice; 339 were subjected to biopsy and 188 served as controls. There were no significant differences in trophoblastic vesicle formation at the four-cell stage between controls (0%) and removal of one or two blastomeres (1% and 7%, respectively) by biopsy (Fig. 1). Biopsy of three (21%) blastomeres from the four-cell mouse embryo resulted in a statistically significant increase in formation of trophoblastic vesicles ($P<$ 0.001; Fig. 1).

There were no significant differences in trophoblastic vesicle formation between controls (0%; no blastomeres biopsied) and one, two, three, or four blastomeres (0%, 1%, 2%, and 9%, respectively) biopsied from eight-cell mouse embryos (Fig. 2). The biopsy of five (75%) blastomeres from eight-

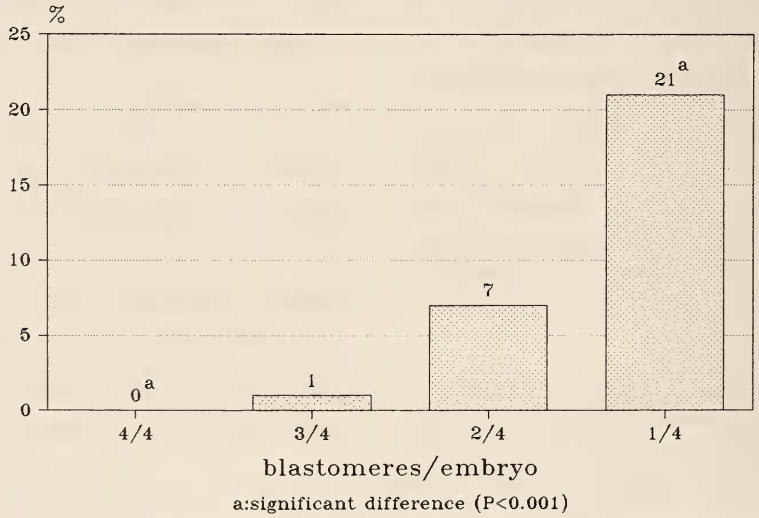

Fig. 1. Trophoblastic vesicle formation in biopsied 4-cell mouse embryos.

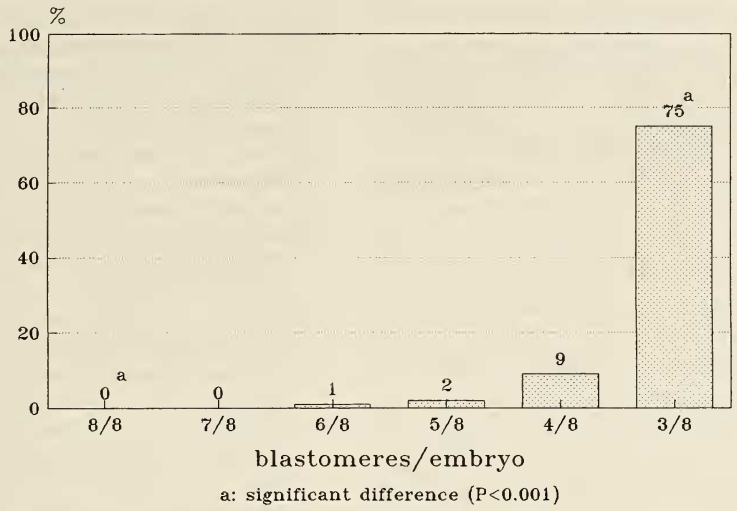

FIG. 2. Trophoblastic vesicle formation in biopsied 8-cell mouse embryos.

cell mouse embryos was found to significantly increase trophoblastic vesicles formation when compared with controls ($P<0.001$; Fig. 2).

DISCUSSION

The present study confirms our earlier report that mouse embryos can be biopsied and have 50% of the cells removed without a significant increase in the formation of abnormal blastocysts [2]. We have previously reported that a maximum of one (25% of embryonic mass) or three (37.5% of embryonic mass) blastomeres can be biopsied from four- or eight-cell mouse embryos without significantly affecting normal development *in vitro* or *in vivo* [2]. Kelly [11] reported that blastomeres from four- to early eight-cell mouse embryos maintain totipotentiality. However, cells from compacted eight-cell mouse embryos will develop into trophoblastic vesicles [8]. This suggests a loss of cellular totipotency in the late eight-cell mouse embryo. It may be that blastomeres from early embryos require a minimal cellular mass (e.g. 50%) to maintain developmental totipotentiality.

Fetal and term development has been established in a number of species, including humans, following biopsy and oviductal or uterine transfer [2, 3, 5, 9, 11, 13]. However, manipulated embryos do not develop *in vivo* as well as non-manipulated embryos [13]. Early post-implantation half embryos have significantly more trophoectodermal cells than ICM [15]. The poor post-transfer development of biopsied pre-embryos can not be solely due to abnormal development (trophoblastic vesicle formation) of the developing embryos.

The poor post-transfer development success of biopsied preembryos may be attributed to bacterial, viral, or immune attack through the violated zona pellucida. Nichols and Gardner [14] reported that damage to the zona pellucida will impair embryo development *in vivo*. They suggested that the intact zona pellucida protects the developing embryo from damage by oviductal compression. However, Cohen [16] reported that improved implantation and clinical pregnancy rates can be achieved following the uterine transfer of partial zona dissection (PZD) of *in vitro* fertilized human embryos. Further studies are required to understand why biopsied embryos do not develop as well as non-manipulated or PZD-manipulated embryos following transfer.

Cell differentiation is first grossly apparent at the blastocyst stage when two distinct cell types are found, the ICM and trophectoderm. Radially polarized blastomeres in 8-cell embryos is the first identifiable stage of cell differentiation in the mouse [17]. Prior to differentiation the embryonic cells must become determined. The process of cell determination is not fully understood as to how or

what influences it to occur. However, several theories exist, these include: (1) epigenetic: dependent upon blastomere position at time of determination-differentiation [11, 18–20]; (2) cell apposition: based on the epigenetic hypothesis, where the differentiation signal arises from the sensitivity of the cell's metabolic activity to the percentage of the cell's surface that is apposed vs. free to the external environment [21]; (3) cell division asynchrony: first division cells are more likely to develop as ICM [11]; (4) cytoplasmic determinants [17]; and (5) cellular interactions [22]. Cosby *et al.* [23] suggested that cell determination occurs at or prior to the eight-cell stage in the developing mouse embryo. The removal of trophoectodermal-detemined but non-differentiated cells may result in the reduction of sufficient quantities of cells required for implantation and subsequent placental formation. Krzyminska *et al.* [6] suggested that the biopsy of embryos might result in fewer cells to form the ICM. However, several studies have reported fetal development following the transfer of trophoectodermal biopsied embryos [12, 13]. There may be a critical number of trophoectodermal cells required for sufficient placental development.

REFERENCES

1 Roudebush, W. E., and Dukelow, W. R. (1991) Embryonic biopsy by cell displacement maintains an intact blastomere without disrupting development. Zool. Sci., **8**: 323–328.

2 Roudebush, W. E., Kim, J. G., Minhas, B. S., and Dodson, M. G. (1990) Survival and cell acquisition rates after preimplantation embryo biopsy: Use of two mechanical techniques and two mouse strains. Am. J. Obstet. Gynecol., **162**: 1084–1090.

3 Handyside, A. H., Kontogianni, E. H., Hardy, K., and Winston, R. M. L. (1990) Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplication. Nature, **344**: 768–770.

4 Monk, M., Handyside, A., Hardy, K., and Whittingham, D. (1987) Preimplantation diagnosis of deficiency of hypoxanthine phosphoribosyl transferase in a mouse model for Lesch-Nyhan syndrome. Lancet, **2**: 423–425.

5 Nijs, M. M., Camus, M., and Van Steirteghem, A. C. (1989) Evaluation of different biopsy methods of blastomeres from 2 cell mouse embryos. Hum. Reprod., **3**: 999–1003.

6 Krzyminska, U. B., Lutjen, J., and O'Neill, C. O. (1990) Assessement of the viability and pregnancy potential of mouse embryos biopsied at different preimplantation stages of development. Hum. Reprod., **5**: 203–208.

7 Fiser, P. S., and Macpherson, J. W. (1976) Development of embryonic structures from isolated mouse blastomeres. Can. J. Anim. Sci., **56**: 33–36.

8 Rossant, J. (1976) Postimplantation development of blastomeres isolated from 4- and 8-cell stage mouse embryos. J. Embryol. exp. Morph., **36**: 283–290.

9 Wilton, L. J. and Trounson, A. O. (1989) Biopsy of preimplantation mouse embryos: Development of manipulated embryos and proliferation of single blastomeres *in vitro*. Biol. Reprod., **40**: 145–152.

10 Dandekar, P. V. and Glass, R. H. (1987) Development of mouse embryos *in vitro* is affected by strain and culture medium. Gamete. Res., **17**: 279–285.

11 Kelly, S. J. (1975) Studies of the potency of the early cleavage blastomeres of the mouse. In "The Early Development of Mammals". Ed. by M. Balls and A. E. Wild, Cambridge University Press, London, pp. 97–105.

12 Monk, M., Muggleton-Harris, A. L., Rawings, E. and Whittingham, D. G. (1988) Pre-implantation diagnosis of HPRT-deficient male and carrier female mouse embryos by trophectoderm biopsy. Hum. Reprod., **3**: 377–381.

13 Summers, P. M., Campbell, J. M. and Miller, M. W. (1988) Normal *in vivo* development of marmoset monkey embryos after trophectoderm biopsy. Hum. Repord., **3**: 389–393.

14 Nichols, J. and Gardner, R. L. (1989) Effect of damage to the zona pellucida on development of preimplantation embryos in the mouse. Hum. Reprod., **4**: 180–187.

15 Rands, G. F. (1986) Size regulation in the mouse embryo. II. The development of half embryos. J. Embryol. exp. Morph., **98**: 209–217.

16 Cohen, J. (1991) Assisted hatching of human embryos. J. In Vitro Fert. Transfer, **8**: 179–190.

17 Johnson, M. H., Chisholm, J. C., Fleming, T. P. and Houliston, E. (1986) A role for cytoplasmic determinants in the development of the mouse early embryo? J. Embryol. exp. Morph., **99**: 97–121.

18 Tarkowski, A. K. and Wroblewska, J. (1967) Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. J. Embryol. exp. Morph., **18**: 155–180.

19 Hillman, N., Sherman, M. I. and Graham, C. (1972) The effect of spatial arrangement on cell determination during mouse development. J. Embryol. exp. Morph., **28**: 263–278.

20 Fleming, T. P. (1987) A quantitative analysis of cell allocation to trophoectoderm and inner cell mass in the mouse blastocyst. Develop. Biol., **119**: 520–531.

21 Wiley, C. M. (1984) The cell surface of the mammalian embryo during early development. In "The Ultrastructure of Reproduction". Ed. by Blerkom J. Motta, Martinus Nijhoff, Boston, pp. 190–204.

22 Dyce, J., George, M., Goodall, H. and Fleming, T. P. (1987) Do trophectoderm and inner cell mass cells in the mouse maintain discrete lineages? Development, **100**: 685–698.

23 Cosby, N. C., Roudebush, W. E., Ye Lian and Dukelow, W. R. (1988) Cell differentiation as determined by micromanipulation of mouse embryos. Biol. Reprod., **38** (suppl. 1): 128.